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13. ABSTRACT *(Maximum 200 Words)*

My purpose is try to identify a plasma membrane protein fingerprint which can be used to distinguish invasive breast cancer cell lines from non-invasive breast cancer lines. The methods included those for purification of plasma membrane proteins , extraction of proteins using urea/thiourea mixture, a standard procedure for 2D PAGE, excision of polypeptide "spots" from gels, trypsin digestion, MALDI TOF mass spectrometry and database searching for protein identity are well-established for this project. Three breast cancer cell lines (MDA-MB435, MDA-MB231 and MCF7) with different metastatic potential have been grown and 2-D maps of plasma membrane proteins have been generated in the laboratory. Computer-assisted analysis of the 2D polypeptide pattern showed that 56% of the polypeptide spots were the same between MCF7 and MDA-MB231 (two non-metastatic Breast Cancer cell lines), only 42% of the polypeptide spots were the same between MCF7 and MDA-MB435 (metastatic vs non-metastatic Breast Cancer cell lines). This differential expression of proteins is being characterized in order to determine which proteins are important to the metastatic condition. A number of proteins from the 2D gels has been identified by MALDI TOF mass spectrometry. Recently, antibody library phage display, has been expanded for further identifying the plasma membrane proteins.

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Vansberg 9-27-1993

PI - Signature Date

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Accomplishments towards Objectives

During the six months that I have worked on this project, progress has been made in numerous areas. With my assistance, many of the proteomics methodologies underwent final optimization and standard operating procedures were adopted. The methods included those for purification of plasma membrane proteins using cationic colloidal silica, extraction of proteins using urea/thiourea mixture and 2D PAGE parameters. In addition, a new method was established for spotting of matrix and peptides onto MALDI targets. This new technique, with its increased sensitivity and peptide yield, greatly advances our ability to identify proteins from 2D gels.

Specific Aim 1

Task 1. This task has not yet been completed. Although we have 3 adherent breast cancer cell lines in-house, we need to obtain the cell line for growth in suspension. The method for purification of plasma membranes using colloidal silica and extraction of the plasma membrane proteins using urea/thiourea has already been well-established in this lab.

Task 2. Since we do not yet have a breast cancer cell line for growth in suspension this task has not been addressed; however, a standard procedure for 2D PAGE of plasma membrane proteins has been adopted and the gel analysis software has been used extensively by the lab.

Specific Aim 2

Task 1. Although this task has not been accomplished, the techniques for excision of polypeptide "spots" from gels, trypsin digestion, MALDI TOF mass spectrometry and database searching for protein identity are well-established in the lab.

Specific Aim 3

Task 1. Three breast cancer cell lines (MDA-MB435, MDA-MB231 and MCF7) with different metastatic potential have been grown in the laboratory. More specifically, MCF7 is representative of a non-invasive mammary carcinoma as the cells retain epithelial morphology, require estrogen for growth and also fail to metastasize when implanted into the mammary fat pad of mice. In contrast, the MDA-MB435 cells exhibit a mesenchymal morphology, grow independently of estrogen and metastasize extensively from the mammary fat pad of mice. The third mammary carcinoma cell line, MDA-MB231, is tumorgenic but non-metastatic.

Task 2. Two-dimensional maps of plasma membrane proteins have been generated from the three breast cancer cell lines. On average, 376 polypeptide spots were resolved on each of the 2D gels. Computer-assisted analysis of the plasma membrane protein 2D polypeptide pattern showed that 56% of the polypeptide spots were the same between MCF7 and MDA-MB231 (two non-metastatic Breast Cancer cell lines), whereas only

42% of the polypeptide spots were the same between MCF7 and MDA-MB435 (metastatic vs non-metastatic Breast Cancer cell lines). This differential expression of proteins at the plasma membrane is being characterized in order to determine which proteins are important to the metastatic condition.

Specific Aim 4

Task 1. This task has been initiated in that MALDI TOF mass spectrometry has been used to identify a number of proteins from the 2D gels of the plasma membrane proteins of metastatic and non-metastatic breast cancer cells.

Note: Specific Aim 4 has recently been expanded to include an additional methodology, antibody library phage display, for identifying the plasma membrane proteins that differ in expression between metastatic and non-metastatic breast cancer cells. This unique methodology will complement and further advance our ability to identify the complex array of plasma membrane proteins that contribute to the metastatic phenotype. In this approach RNA is extracted from the spleens of mice that have been immunized with the purified plasma membrane proteins of a cell line of interest. A cDNA library is then constructed by reverse transcription of the extracted RNA and a mouse Immunoglobulin G scFv (single chain variable Fragment) library is generated from this cDNA library. Finally, a phage display scFv library is constructed. The antibodies that display on the surface of the phage are then screened to identify the specific antibodies that bind the plasma membrane proteins immobilized on the blots of 2D gels. Similarities and differences between metastatic and non-metastatic breast cancer cell lines can be observed by identifying the differential binding of phage to the blots. The phage display strategy will be a powerful asset for obtaining antibodies to multiple proteins on the blots of 2D gels. In combination with the identification of these proteins by mass spectrometry, it is likely that I will be able to identify and raise antibodies to dozens if not hundreds of plasma membrane proteins at one time.

I am currently working out the logistics of the "phage display" approach using an endothelial cell line with known cell-surface markers as a positive control situation. Mice have been immunized and the sera evaluated by Enzyme-linked immunosorbent assay (ELISA) and by Fluorescence-assisted cell sorting (FACS). In both cases, the immune sera showed a positive response towards known endothelial cell-surface markers, whereas pre-immune sera did not. RNA has been isolated from the spleens of the immunized mice, a panel of IgG scFv library has been generated and a phage display scFv antibody library is currently being constructed.